EXHIBIT 1

Review TRENDS in Biochemical Sciences Vol.31 No.9

Huntington's disease: seeing the pathogenic process through a genetic lens

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Thirteen years ago, the culmination of genetic rather than biochemical strategies resulted in the identification of the root cause of Huntington's disease: an expanded CAG trinucleotide repeat that leads to an elongated polyglutamine tract in the huntingtin protein. Since then, biochemical and cell biological attempts to elucidate pathogenesis have largely focused on N-terminal polyglutamine-containing huntingtin fragments. However, continued application of genetic strategies has suggested that the disease process is, in fact, triggered by the presence of expanded polyglutamine in intact huntingtin. An increased emphasis on the earliest presymptomatic stages of the disease, facilitated by incorporating genetic lessons from human patients into the search for biochemical targets, could provide a route to a rational treatment to prevent or slow the onset of this devastating neurodegenerative disorder.

An exceptional opportunity to prevent neurodegeneration

Huntington's disease (HD) has been a flagship for the study of inherited neurological disease, from initial chromosomal localization of the gene without any prior understanding of its nature, through identification of the molecular defect without knowledge of the gene or its function, to characterization of pathogenesis using gene-based models. Unlike other common neurological disorders, HD has a single cause in all patients, which enables investigators to focus on a single fundamental disease mechanism. Genetics has made it possible to approach that mechanism from its initial stages, without having to work backwards from end-stage pathology. This feature of HD contrasts with the heterogeneous etiologies of well-known disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis that force therapeutic efforts to be trained upon shared biochemical events that occur late in each disease process. It is notable, however, that, even in these heterogeneous disorders, much of the current knowledge is owed to the identification of genetic defects in the small proportion of cases with evident genetic etiology.

The single starting point for HD pathogenesis offers the special opportunity for discovering a rational therapy that blocks the initial triggering event or its immediate biochemical consequences. However, despite this unique advantage, much HD research has not capitalized on it, being focused instead on cell biological and biochemical events that occur relatively late in the disease process, particularly the formation of insoluble aggregates of truncated protein. Recently, biochemical and other phenotypes have been described that are caused by the expression of endogenous full-length huntingtin and occur long before the appearance of huntingtin fragments or insoluble aggregates. These findings, combined with the capacity to apply genetic criteria to dissect the disease mechanism, suggest that it is timely to increase the focus of experimentation on the earliest stages of pathogenesis – it is these stages that offer the promise understanding the presymptomatic disease state and could lead to targeted therapies that prevent disease onset.

Here, we consider the current status of the understanding of HD pathogenic process prior to late-state-stage neurodegeneration, as guided by genetic studies in HD patients and accurate genetic models.

Clinical and neuropathological features of HD

HD patients are typically recognized by their peculiar writhing movements, but they also suffer behavioral and intellectual deficits [1]. Disease manifestations can begin at any time in life, although the vast majority of cases display onset in middle-age. HD symptoms are associated with a distinctive underlying neuropathology that starts with the death of GABAergic medium-sized spiny projection neurons in the caudate nucleus (a tail-shaped region bulging into a lateral ventricle of the brain) and then progresses to neurons in other brain regions. Motor symptoms begin subtly as minor spontaneous movements that progress to continuous, involuntary jerky movements, giving way to eventual rigidity. At the time of first motor onset, it is estimated that 20–30% of the caudate nucleus neurons have already been lost. Psychiatric symptoms, including chronic depression, irritability, impulsiveness and aggression, are variable and sometimes precede motor onset by years. Intellectual decline occurs with disease progression, which further contributes to the loss of capacity to function in daily life. Death in HD typically occurs ~15 years after motor onset due to complications of the disorder, such as aspiration pneumonia.

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Available online 10 July 2006.

  doi:10.1016/j.tibs.2006.06.009

Inheritance of the HD genetic defect

HD has traditionally been defined, like other diseases, in terms of its clinical and neuropathological description. However, these are only evident in mid-life and actually represent relatively late aspects of a process that is encoded in the DNA from conception. All cases of HD are caused by inheritance of an expanded CAG trinucleotide repeat. This repeat normally comprises 6–34 CAG units, but expansion beyond this range causes onset of disease symptoms within a normal life span [1]. Interestingly, when a CAG repeat is in the pathogenic range (>34 CAG units), the number of CAG units inherited is meiotically unstable: in most parent-to-child transmissions, the child's CAG repeat is one or a few units longer or shorter than the parent's (i.e. a mutation rate that approaches 1). Inheritance of the *HD* gene from fathers might occasionally show much larger size jumps due to particular instability of the *HD* CAG repeat in spermatogenesis in some males.

The length of the *HD* CAG repeat is the primary determinant of the age at which clinical symptoms will appear. An increase in CAG length correlates with a decrease in age of onset of neurological symptoms, with the longest CAG repeats causing juvenile-onset HD (Figure 1). This is an extremely strong functional relationship, with the CAG-repeat length alone accounting for 70% of the variance observed for age at onset [2]. The remaining variance, which accounts for a range of onset ages of ±19 years around the mean for most CAG-repeat lengths, is also heritable (proportion of phenotypic variance attributable to genetic variance, $h^2 = 0.56$), indicating the actions of modifier genes [2,3]. Interestingly, in the same dataset, there is no correlation between CAG length and duration of disease from neurological onset to death (Figure 1). Similarly, in a recent extensive study of clinical phenotypes in a large HD cohort, it was found that there is only a small effect of CAG length on progression of overall neurological signs, motor impairment and cognition, but no significant effect on progression of chorea or activities of daily life [4]. These and other studies of progression (see Ref. [4] and references therein) indicate that, although the process that initiates HD pathogenesis is highly dependent on CAG-repeat length, the mechanisms that lead from overt symptoms to eventual death are not. Thus, the search for treatments aimed at blocking disease progression might require fundamentally different approaches from the search for therapeutics to prevent disease onset.

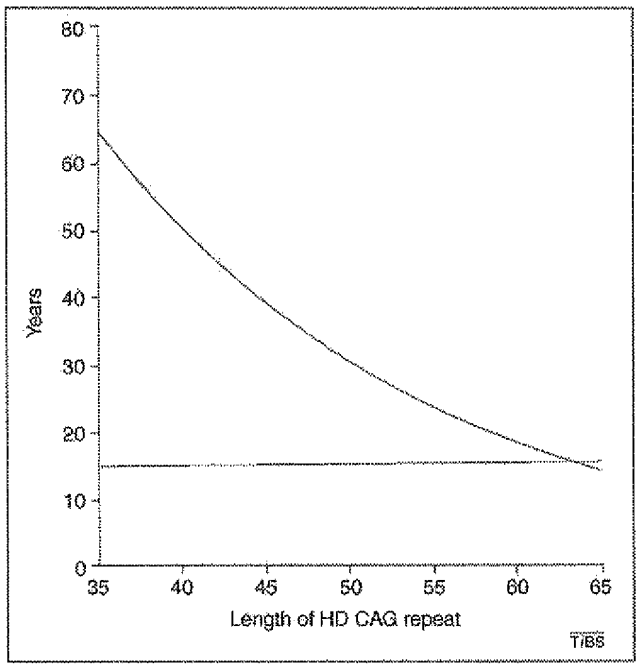

Figure 1. Correlation of HD CAG-repeat length with age at onset. Best-fit curves for age at neurological onset (red) and duration of disease from onset to death (blue), plotted against CAG-repeat length for the expanded mutant allele from Huntington disease (HD) patients. Age at onset is strongly correlated with the CAG-repeat length ($r^2 = 0.54$; $p < 0.001$), whereas duration of disease shows no correlation with the CAG-repeat length, suggesting that factors independent of the original trigger of pathogenesis predominate after onset of HD to determine rate of progression. Based on the data from Ref. [75].

Two special circumstances in which this striking relationship between CAG-repeat length and age at onset has been investigated are also revealing concerning both the inheritance and functional characteristics of the mutation. First, this relationship, together with the occasional larger increases in repeat size seen in paternal transmissions of the disease gene, explains the previously enigmatic observation that most juvenile-onset HD patients have inherited their *HD* gene from a father. Second, although most HD patients possess a single copy of the genetic defect, rare cases have been reported whereby an individual has inherited two expanded copies, one from each affected parent. Investigations of these rare 'HD homozygotes' show that neither the presence of two copies of an HD expanded CAG repeat nor the absence of a normal CAG repeat alters significantly the age at neurological onset predicted by the longer of the two expanded disease alleles [5–8]. Thus, at least with respect to age at neurological onset, HD exhibits phenotypic dominance.

The HD genetic defect in context

The CAG repeat is located in the coding sequence near the 5′ end of the *HD* gene. It encodes a variable length polyglutamine tract beginning 18 amino acids from the N terminus of the large (>3100 amino acid) huntingtin protein. Huntingtin is expressed widely from conception in both neuronal and non-neuronal tissues, and, within the brain, is not limited to the neurons that are vulnerable in HD. Indeed, this pattern in which neuronal susceptibility is not explained by preferential protein expression parallels the observations in a series of other inherited neurodegenerative disorders in which expanded CAG repeats encode lengthened glutamine tracts in different proteins (Figure 2). However, in each of these disorders, a different neuronal population is the primary target. For example, in spinocerebellar ataxia 1, the glutamine tract is within the 815 amino acid ataxin 1 protein. Despite being expressed in both medium spiny neurons in the caudate and Purkinje cells of the cerebellum, this mutant protein leads to primary loss only of the latter neurons. Similarly, huntingtin is expressed in both neuronal populations but the mutant protein leads to primary loss of medium spiny neurons. The striking similarities and notable differences in these neurodegenerative disorders suggest a mechanism by which the effect of the altered

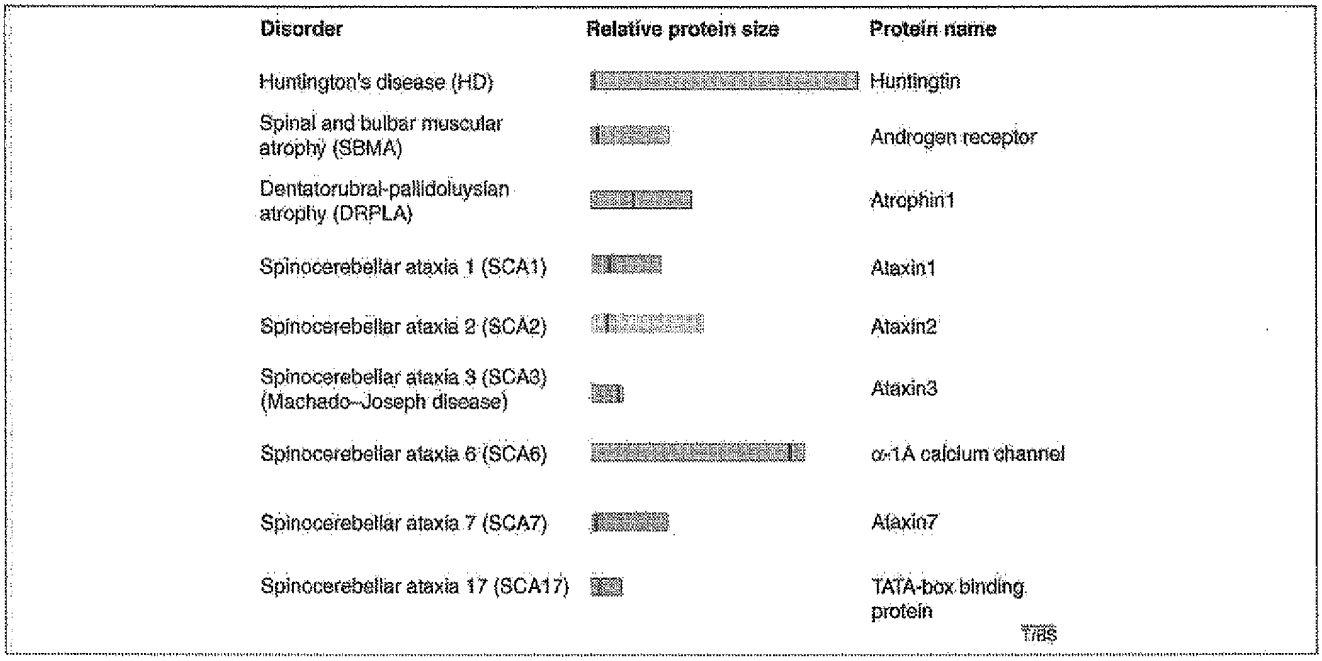

Figure 2. Protein context of the polyglutamine expansion determines which neuronal cell populations are the most vulnerable. Shown is the location of the polymorphic polyglutamine tract in nine different protein contexts (drawn to scale) that, when expanded, causes the specific loss of neurons from different brain regions and lead to distinct inherited neurodegenerative disorders.

polyglutamine combines with its specific protein context to trigger the characteristic pathology in each different disorder.

How does the HD genetic defect kill neurons?

The toxic effects of the *HD* polyglutamine tract have most often been studied within a small N-terminal fragment (hereinafter termed 'h') of the huntingtin protein [9,10]. This approach has been spurred by the observation in post-mortem HD brain of the h in inclusions within remaining neurons [11]. In a wide variety of model systems, including both neuronal and non-neuronal cells in culture, yeast, nematode, fruit fly and rodents, expression of h leads to formation of intracellular inclusions in the cytoplasm and/or nucleus. The correlation of inclusions with cellular toxicity has been extremely variable, with some studies reporting toxic effects and others reporting protective effects [12,13]. This extensive literature has been reviewed often. However, a recent careful quantitation of the time course of inclusion formation and cell death in primary striatal neurons expressing h shows that such inclusion formation is not the source of polyglutamine-mediated toxicity in HD [14]. This has been supported by two other reports in which lentivirus-driven or HD-promoter-driven h produced intraneuronal inclusions without correlation with neuronal loss [15,16].

In contrast to h fragments with polyglutamine lengths in the normal range, those with polyglutamines in the pathogenic range rapidly form inclusions in model systems and aggregated insoluble amyloid *in vitro*. This supports the suggestion that the expanded polyglutamine confers an altered physical property on the protein. An elegant series of studies has shown that this conformational property is dependent on both the polyglutamine tract and its surrounding sequence context [17–19]. Their studies suggest that amyloid formation is nucleated at the level of the single molecule by a thermodynamically unfavorable misfolding that leads to formation of an aggregate structure with alternating elements of extended chain and turn. Some of the predictions from these studies have been reproduced in cultured cells that express variants of the polypeptide encoded by exon 1 of the 67-exon *HD* gene [20]. These types of experiments, combined with the HD genotype–phenotype relationship described, have led to the view that HD is a conformational disorder in which the pathogenic pathway is triggered in some manner by protein misfolding and its consequences.

Whereas an altered physical property is readily measured in the context of the h fragment, its effects on the full-length huntingtin protein are not as well understood. For example, Cong *et al.* [21] have shown that distinct differences on native gels between soluble h with glutamine tracts in the mutant and normal size ranges are abolished in larger fragments that included more of the downstream huntingtin sequence. These results suggest that the huntingtin sequence dampens the acute effects of the conformational difference between mutant and normal-sized polyglutamine tracts [21]. As expression of full-length mutant huntingtin occurs throughout life and precedes the detection of h in human patients, the possibility that pathogenesis is triggered via a novel property conferred on the huntingtin protein has been tested in a variety of mouse models.

What is the effect of polyglutamine on huntingtin?

A route used by several laboratories to test the consequences of an elongated polyglutamine tract in intact huntingtin has been to create *Hdh* (the mouse *HD* gene

ortholog) CAG knock-in lines of mice as true genetic models of HD. These lines express mutant huntingtin with 50–150 glutamines from the endogenous mouse *Hdh* promoter in a manner comparable to the expression of mutant huntingtin in HD patients [22]. These mouse models exhibit a variety of abnormalities that are detected at every level, from the biochemical, molecular and cellular level to phenotypes that can be measured in the whole animal. As expected based upon HD in humans, changes are first apparent in the striatum (i.e. the caudate nucleus, putamen and globus pallidus). Molecular phenotypes include: (i) increased levels of the ribosome signaling protein Rrs1; (ii) an altered conformation or accumulation of huntingtin in the nucleus of medium-sized spiny neurons; (iii) striatal *Hdh* CAG-repeat instability; (iv) altered enkephalin mRNA level; (v) altered Ca^{2+} sensitivity of striatal mitochondria; and (vi) altered intracellular signaling affecting at least protein kinase A, protein kinase B (Akt), glycogen synthase kinase 3β (GSKβ), and serum- and glucocorticoid-inducible kinase (SGK) pathways [22–25]. Abnormal behaviors such as nocturnal hyperactivity manifest at a young age, well before signs of overt neuronal cell pathology or neurodegeneration [22]. At much older ages, h, insoluble amyloid, and intranuclear and cytoplasmic inclusions become evident. As is the case for neurological onset in humans, the onset of these phenotypes is hastened by longer polyglutamine-repeat lengths and is more sensitive to polyglutamine length than to huntingtin dosage. In animals with the longest glutamine tracts, age also brings striatal atrophy, loss of medium-sized spiny striatal neurons, gait deficits and mildly decreased survival. Notably, homozygotes for the knock-in alleles have a slightly earlier onset of these phenotypes than heterozygotes. At first glance, this seems to differ from the phenotypic dominance observed in humans for age at neurological onset. However, this small difference of a matter of weeks might also manifest in human HD patients but might be undetectable, being overwhelmed by the normal ±19-year variation around the mean for neurological onset.

An alternative approach has involved the analysis of YAC72 and YAC128 transgenic mice, created with modified *HD* 4p16.3 genomic DNA YAC transgenes [26–32]. These mice ubiquitously express copies of human huntingtin from human *HD* promoter elements. The YAC128 mice have recently been shown to exhibit a similar striatal-specific huntingtin nuclear localization phenotype to the knock-in models. As in *Hdh* CAG knock-in mice, this coincides with onset of early behavioral abnormalities and is also followed much later by intranuclear inclusions and striatal-cell degeneration. More dramatic effects of mutant huntingtin have recently been achieved in a tetracycline-off conditional PrP-tTA-6/iFL148Q transgenic line, in which a prion promoter drives conditionally regulated expression of human huntingtin with 148 glutamine residues from a full *HD* cDNA transgene [33]. Transgene expression from the prion promoter is highest in regions other than the striatum. These mice exhibit mutant h and inclusions in regions beyond the striatum, and early death [33]. It will now be important to determine whether inappropriate regulation of mutant huntingtin leads to early presymptomatic disease phenotypes, and in which cell types.

Early presymptomatic effects of properly regulated mutant huntingtin have also been studied in cell culture HD models. Primary striatal cell cultures and clonal immortalized striatal cells derived from *Hdh* CAG knock-in mice and from YAC transgenic mice exhibit a variety of phenotypes comparable to the early striatal-specific phenotypes seen in the mice, but do not manifest h fragments or inclusions [34]. Thus, the accurate expression of intact mutant huntingtin in YAC transgenic and *Hdh* CAG knock-in mice has revealed early phenotypes long before those associated with symptomatic HD, thereby, providing models for the presymptomatic and early symptomatic disease period. The early phenotypes first manifest in striatal neurons, confirming the special vulnerability of this cell population. In addition, they occur in the absence of overt h, indicating that, although aggregate formation might have a role downstream, it does not trigger the disease process, which, instead, begins with an effect of the intact mutant huntingtin.

The HD pathogenic process in humans

The existence of a protracted pathogenic process in humans, as seen in mice, is increasingly apparent from brain imaging and neuropsychological and cognitive studies of presymptomatic individual, made possible by HD CAG testing. These have revealed both morphometric and functional changes in the brain that occur prior to the onset of marked neurological symptoms [35–45]. It is not known how early in life these differences begin, but they have often been presumed to result from a direct effect of mutant huntingtin on a neuron-specific target. Interestingly, it is now also being recognized that presymptomatic individuals display abnormalities due to mutant huntingtin in non-neuronal tissues such as skeletal muscle, fibroblasts, platelets, blood-nucleated cells and cultured lymphoblasts [46–53]. These findings present an alternative model for the HD disease process, suggesting that the effect of mutant huntingtin might alteration of the fundamental state of any cell in a CAG-length-dependent manner. Although most cells have the capacity to survive the CAG-length-dependent state, striatal medium-sized spiny neurons eventually exhibit dysfunction and succumb owing to some peculiarity that makes them unable to cope (Figure 3). In this model, striatal specificity lies not in the nature of the direct target of mutant huntingtin, but in the special physiology of striatal neurons that makes them vulnerable. Deciphering the nature of this striatal specificity could provide clues to treatment that cannot be identified using other cell types.

Impact of polyglutamine on huntingtin normal function

Huntingtin deficiency is lethal both in mouse embryogenesis and in adult cells and, although increasing huntingtin levels might help cells to survive certain stresses, the actual function(s) of the protein is not known [54]. The existence of HD homozygote patients with no normal *HD* gene and whose age at neurological onset is comparable to equivalent HD heterozygotes has made it clear that the expanded CAG does not greatly impair the essential developmental function(s) of huntingtin. Neither does it cause onset by a mechanism that can be rescued by one

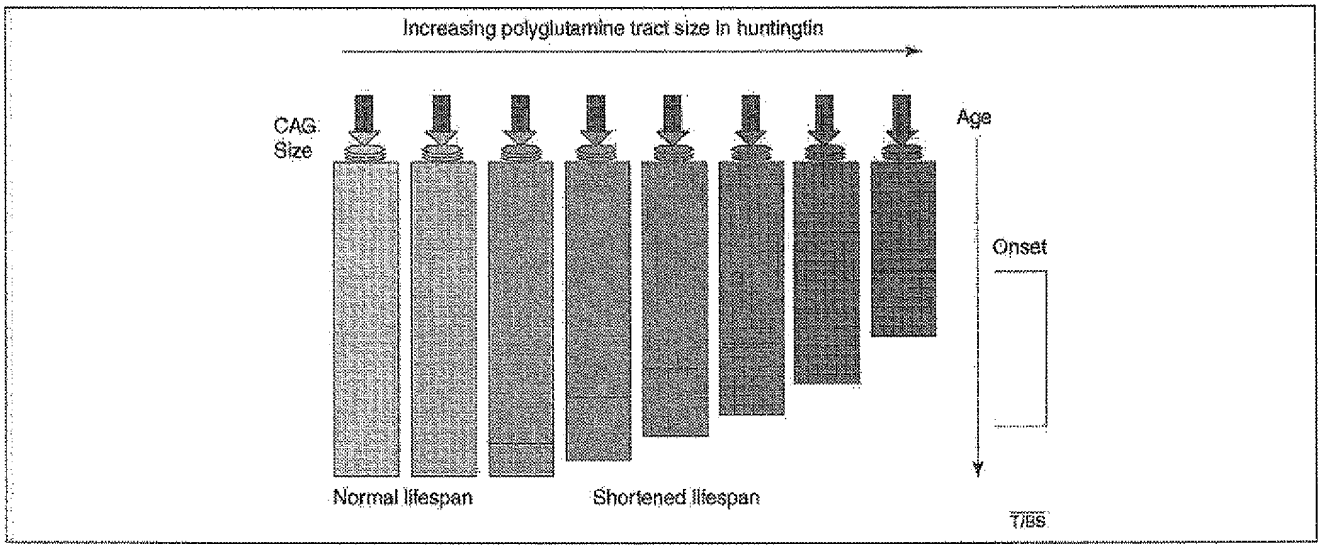

Figure 3. A quantitative trigger might determine a constitutional physiologic state. The schematic diagram depicts a model for a hypothetical quantitative effect of the *HD* CAG repeat that might manifest in both non-pathogenic and pathogenic size ranges in many cell types throughout life, as suggested by the quantitative effect of *HD* CAG size on the cellular ATP:ADP ratio [53]. The blue rectangle above each box represents huntingtin and the arrow at its base represents its polyglutamine tract, the increasing color intensity of which indicates increasing polyglutamine length. Huntingtin interacts with an unknown cellular target (denoted by the octagon) with intensity or frequency of interaction also increasing with polyglutamine length (denoted by increasing color intensity). This huntingtin–target interaction determines a physiologic state (denoted by the large rectangle) that lasts throughout life and is dependent on the glutamine-tract length. The physiological state becomes increasingly intense (denoted by red color intensity) with increased glutamine size within the normal size range (<35 CAGs), but is not sufficient to disable striatal neurons, does not lead to onset of HD and supports a normal lifespan. CAG lengths of 39 and higher produce a progressively more intense physiological state with which striatal neurons cannot cope, thereby leading to onset of overt clinical symptoms (black vertical bars) at progressively earlier ages. Premature death ensues after a course of ~15 years, but is not influenced by repeat size. Intermediate glutamine tracts (35–38 CAGs) might cause onset late in life, <15 years before the average normal lifespan.

allelic equivalent of the normal protein. One report suggests that HD homozygotes display more rapid progression after onset, indicating that wild-type huntingtin might possess a protective activity [6]. However, as neither the progression nor the duration of illness after neurological onset is strongly correlated with CAG length, this protective activity might act on processes secondary to the initial trigger and to the neuronal dysfunction that causes disease onset.

Still, the fact that the disease process triggered in HD is different from that triggered in other polyglutamine disorders indicates that some aspect of the structure, binding partners, subcellular localization or activity of huntingtin is crucial to this specificity. Huntingtin is composed largely of consecutive HEAT repeats, which ate ~38 amino acid degenerate motifs that are named for their presence in huntingtin, elongation factor 3, regulatory A subunit of protein phosphatase 2A and TOR1 (target of rapamycin 1) [55,56]. Each HEAT repeat consists of two α-helical domains separated by a short linker. X-ray crystallography of other HEAT repeat proteins, such as β-importin and the regulatory A subunit of protein phosphatase 2A has revealed a stacking of consecutive HEAT repeats that forms a flexible solenoid-like structure. A recent preliminary biochemical characterization of recombinant full-length human huntingtin is consistent with this model, with huntingtin being a much larger protein that might form an elongated superhelix [57]. Based on its HEAT-repeat structure, huntingtin has been suggested to function as a scaffold, organizing members of dynamic complexes for transport and/or activity [56]. Huntingtin interacts with membranes and a wide range of other proteins that represent many different cellular functions, and is subject to a variety of post-translational modifications [54,58,59]. It shuttles between cytoplasmic and nuclear compartments and subsets of the protein are differentially detectable by immuno-staining, which indicate different conformations or epitope availability in different locations. Huntingtin has been implicated in facilitating a variety of cellular processes, including transcriptional regulation, mRNA processing, vesicular transport, and organellar location and morphology.

Interestingly, the polyglutamine segment is dispensable because its removal from mouse huntingtin leads to animals that are fully viable [60]. However, they develop subtle motor and behavioral differences from wild-type mice, suggesting that the glutamine tract might modulate a normal function of huntingtin. Cultured fibroblasts from these mice display elevated ATP levels. Notably, mouse striatal cells from *Hdh* knock-in mice with an elongated glutamine tract show reduced ATP levels, which could be an effect of polyglutamine length. Examination of human lymphoblasts representing both the pathogenic and non-pathogenic size ranges has revealed a clear correlation between polyglutamine length in endogenous huntingtin and the cellular ATP:ADP ratio [53]. These findings on the effect of the polyglutamine tract combine to implicate huntingtin either directly or indirectly in regulation of energy metabolism, which might also be consistent with the recent description of an effect of huntingtin dosage on body weight in mice [26].

Modifying the pathogenic process

The understanding of the structure and function of huntingtin is not yet sufficient for small-molecule drugs

to be targeted with certainty to the huntingtin-specific trigger of pathogenesis. Continued delineation of the earliest events in pathogenesis and further examination of the structure and function of huntingtin will be required. However, both can be informed by the identification of pharmacological and genetic modifiers of the disease process either in model systems or in human patients. Many drug screens have been performed in cellular models, typically with different results in each, confirming that the polyglutamine has different consequences in different contexts and different cell types [61]. This suggests that the many cellular assays do not, as a group, capture the same fundamental mechanism. As a conformational property is thought to trigger the pathogenic process, several drug screens have been aimed at altering this property *in vitro*. Testing of these compounds in mouse models represents the current best hope for a drug that blocks the beginnings of pathogenesis. It is hopeful that some compounds identified in this manner rescue a phenotype caused by intact mutant huntingtin in cultured striatal cells from *Hdh* knock-in mice [62]. In the absence of a small molecule treatment, blocking the pathogenic trigger might require manipulation of endogenous mutant huntingtin expression via siRNA, intrabodies or other techniques [63–66].

Another approach that might provide clues to presymptomatic treatment is the search for those genes that reveal the heritable variance of age at onset that is not due to the HD CAG repeat. Several potential genetic modifiers have emerged from candidate association studies in HD patients, of which only the gene encoding the GluR6 subunit of the kainate-type glutamate receptor (*GRIK2*) and the gene encoding ubiquitin C-terminal esterase L1 (*UCHL1*) have yet been replicated in more than one population [67–71]. These modifiers, which might impact by altering glutamate-mediated signaling and huntingtin clearance, respectively, explain a relatively small portion of the variance in age at onset. However, when confirmed, even modifiers with a small but significant impact can provide potential drug targets because they have been validated as having a measurable effect on HD pathogenesis in human patients. Candidates have also been tested in the mouse, where DNA repair has emerged as a potential drug target [72,73]. Another hopeful direction is to identify genetic modifiers in humans by unbiased genetic linkage and association studies. An initial linkage scan by an international consortium of HD investigators has implicated several chromosomal regions as potentially harboring common modifiers of HD [74]. Although this strategy might take longer than candidate searches, it has the potential to yield the unexpected and cast entirely new light on the pathogenic process.

Concluding remarks

The homogeneous genetic nature of HD offers a tremendous opportunity to identify and block the very earliest stages of the disease process, before widespread neuronal dysfunction occurs. Although examination of the final stages of dysfunction might provide functional drug targets to treat particular phenotypes, genetics has provided the means for describing the characteristics of the initial trigger mechanism and its consequences in model systems. Rational treatments based on the biochemical nature of the trigger and/or early steps in the disease process could prevent all subsequent phenotypes from developing. However, identification of valid biochemical targets at which to aim these rational therapies will require paying close attention to the information provided by the use of genetic strategies in human clinical studies, in experimental models and in drug development.

Acknowledgements

The authors' work on Huntington's disease is supported by NIH grants from the NINDS: NS16367 (MA Huntington's Disease Center Without Walls) and NS32765, and from the NLM: LM008748, by the Hereditary Disease Foundation, and the Huntington's Disease Society of America. The authors are members of the HDSA Coalition for the Cure Team 1 (Mitochondria and Energy Metabolism) and Team 5 (Huntingtin Normal Function).

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